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October 23, 2008

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THIS DOCUMENT SUBMITTED ELECTRONICALLY

RE: Comments on NTP Report on Carcinogens, Styrene Expert Panel's Listing Status for Styrene & Scientific Justification

Dear Dr. Lunn:

The Styrene Information & Research Center¹ (SIRC) is submitting these comments to the National Toxicology Program (NTP) in response to its *Federal Register* notice requesting public comments on Expert Panel's Recommendation on the Listing Status for Styrene in the *12th Report on Carcinogens (RoC)*, and the Scientific Justification for the Recommendation. 73 Fed. Reg. No.174, 52059 (September 8, 2008)

We are submitting the scientific comments herein because ***SIRC does not believe the Styrene Expert Panel's justifications adequately support their proposal that styrene be listed as "reasonably anticipated to be a human carcinogen" in the 12th RoC, and do not reflect a thorough evaluation of the data:***

1. A complete and balanced review of the available human data does not support the Expert Panel's conclusion of "limited evidence of cancer in humans."
2. A complete and balanced review of the available data provides only limited evidence of carcinogenicity in animals, based on no evidence in rats and limited evidence in mice.
3. The mode of action data indicate that tumors found in mice exposed to styrene are not relevant for human risk assessment.

¹ The Styrene Information and Research Center's (SIRC's) mission is to evaluate existing data on potential health effects of styrene, and develop additional data where it is needed. SIRC has gained recognition as a reliable source of information on styrene and helping ensure that regulatory decisions are based on sound science. For more information, visit <http://www.styrene.org>.

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This letter also reiterates issues which SIRC has raised in previous communications to NTP:

1. The Expert Panel generated a new and unpublished epidemiological finding when it concluded there was an association between styrene exposure and non-Hodgkin lymphoma (NHL), based on the University of Alabama study by Delzell et al. (2006). Delzell et al. did not analyze for exposure-response trends for NHL and styrene, or for NHL-CLL combined; therefore, this was a new, unpublished analysis by the Panel.
2. As far as SIRC is aware, it appears that the Panel did not generate a statistical analysis in support of this conclusion.
3. This new and unpublished analysis has not been independently peer reviewed.
4. This new and unpublished analysis was not available for public comment, as it was not included in the Styrene Background Document.
5. A SIRC-sponsored independent "Blue Ribbon Epidemiology Panel" is currently reviewing the collective human epidemiological data set, including the Panel's reanalysis, which could provide the needed external peer review of the Panel's conclusions. The Blue Ribbon Panel's report is expected to be submitted to NTP on December 22, 2008. SIRC will have no advance notice of its contents.
6. SIRC has requested that NTP extend the public comment period on the Panel Recommendations in order to have the "Blue Ribbon Panel's" report accepted as a public comment.

Additionally, SIRC's comments cover ongoing concerns SIRC has with the *RoC* process itself:

1. SIRC's comments to NTP and to the Expert Panel have not been reflected in the NTP documentation on styrene.
2. SIRC's prior comments pointed out significant limitations in the Styrene Background Document; these limitations have yet to be resolved.
3. We are concerned that SIRC's comments and concerns will not receive adequate attention during the subsequent stages of the styrene *RoC* review process.

Based on SIRC's experiences with the *RoC* process for styrene thus far, SIRC believes the review process will fail to address SIRC's legitimate concerns and, at the end of the day, be scientifically unsupportable.

SIRC is submitting these comments in protest, due to (1) the absence of key data regarding a critical new Expert Panel analysis that, if it exists, has not been made available to the public and, (2) the fact that these new data and analysis by the Expert Panel has not been peer reviewed. SIRC has petitioned NTP in writing to provide these underlying analyses and to extend the October 23rd comment period, in order to adequately address these new data, but no data has been received by SIRC to date, and the comment period has not been extended.

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The data in question are the supporting reanalyses by the Expert Panel of data from an epidemiology study in which the original study authors reported the data were inconclusive, but which through its own reanalysis, the Expert Panel concluded supported a finding of increased cancer incidence. This new determination was a key factor the Expert Panel cited in support of its recommendation to list styrene in the RoC, yet it has not been peer reviewed, was not included in the Background Document, and therefore was not open to public comment.

SIRC's detailed comments on the Panel's conclusions, as well on the issues of concern just summarized, follow.

1 - Scientific Comments on Expert Panel Recommendation and Justification

Comments on Expert Panel Conclusions on Human Cancer Studies

From the Expert Panel Report:

"The expert panel concluded that there was limited evidence for the carcinogenicity of styrene in humans, although some members were of the opinion that the epidemiological data provided sufficient evidence. The strongest evidence for cancer in humans is the association between styrene exposure and non-Hodgkin lymphoma (NHL). This evidence comes from the Delzell *et al.* (2006) analysis in the styrene-butadiene industry and the Kogevinas (1994a) study in the reinforced plastics industry. In the Delzell study there was an exposure-response relationship for NHL and NHL plus chronic lymphocytic leukemia (CLL) that was not attenuated by control for butadiene and only mildly attenuated by control for dimethyldithiocarbamate (DMDTC) (which may not have been appropriate to control for). It is very unlikely that such a strong exposure-response trend could be due to chance, bias, or confounding. These findings are supported by increases in RR for all lymphomas with time since first exposure and estimated average exposure in the multiplant-cohort studied by Kogevinas. Many of the smaller studies found excesses in lymphohematopoietic cancers, although these were not statistically significant. They provide further evidence of consistency in the risk. In addition, there is some evidence for increased risk of pancreatic and esophageal cancer. It should be noted that increased risk of solid tumors was not evaluated in many studies. Studies in workers have provided evidence for DNA adducts and chromosomal aberrations in lymphocytes of styrene-exposed workers which supports a genotoxic mechanism for styrene."

Comments:

The data do not support the Expert Panel's conclusion of "limited evidence of cancer in humans."

The NTP Expert Panel concluded that the styrene-butadiene rubber worker (SBR) cohort provides the strongest evidence of increased cancer in humans exposed to styrene. They further stated that based on increased non-Hodgkin's lymphoma in SBR workers, there was at least limited evidence of styrene-related increased cancer in humans.

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A. By this statement, they have implied that the evidence of cancer from styrene in reinforced plastics and composites (RPC) is not convincing. This agrees with the conclusions of the Harvard Panel (Cohen et al., 2002) and the EU (EU, 2007). The IARC Review in 2002 concluded there was limited evidence of cancer among RPC workers based on combined lymphomas and leukemias. The NTP Expert Panel indicated that these individual cancers should not be combined because they are different diseases, arise from different stem cells and by different modes of action. Therefore, the IARC conclusion was not appropriate.

The RPC studies (Kogevinas et al., 1994 [Kolstad et al., 1994 is a subset of this study]; Ruder et al., 2004; Wong et al., 1994) involve more than 60,000 workers with average exposures up to 200 ppm. NHL was not significantly increased in any of the three studies based on duration of exposure, cumulative exposure, average exposure, or time since first exposure. Although the NTP Expert Panel cited increased relative risks for total lymphomas with time since first exposure and average exposure in the Kogevinas study, there was no increase in NHL based on these measures of exposure. Further, there was no increase in either NHL or total lymphomas based on duration of exposure or cumulative exposure in this study.

As expressed in comments on the draft styrene document submitted to the Styrene Docket by Dr. Lorenz Rhomberg, there are no consistent increases in cancer risk in RPC workers. In fact, there are twice as many significant decreases in cancer risk as increases.

B. The NTP Expert Panel assert that there is increased risk of NHL (and NHL-CLL combined) caused by styrene and not by butadiene in the SBR cohort. They said "In the Delzell study there was an exposure-response relationship for NHL and NHL plus chronic lymphocytic leukemia (CLL) that was not attenuated by control for butadiene and only mildly attenuated by control for dimethyldithio-carbamate (DMDTC) (which may not have been appropriate to control for)." ***As noted in the comments by Dr. Delzell provided to NTP by SIRC on October 21, 2008, there was no statistical analysis in Delzell's' University of Alabama (UAB) report, or manuscripts, for exposure-response trends for NHL or NHL-CLL and styrene.***

Further, the Expert Panel said "It is very unlikely that such a strong exposure-response trend could be due to chance, bias, or confounding." This is a controversial statement. ***The Panel provided no basis for this conclusion.*** There was NOT a strong exposure-response. None of the values for styrene after adjustment for butadiene were significantly different from unexposed workers and were not different from values for butadiene adjusted for styrene.

Dr. Delzell has pointed out that exposure to butadiene is a complicating factor in interpreting effects of styrene in the SBR workers. While the UAB group found little evidence of an association between butadiene and NHL, butadiene was positively associated with CLL.

In addition, the elevated RRs for NHL and CLL-NHL among workers with nonzero exposure to styrene reflect, to some extent, unexplained and substantial deficits of

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deaths from NHL and CLL-NHL in the styrene-unexposed group. These deficits were seen when the workers unexposed to styrene were compared to the general population at large (Delzell et al., 2006, tables 21 and 22). The deficit observed in this “external” comparison was statistically significant for CLL-NHL, based on 2 observed compared to 8.1 expected deaths during the time period 1968-1998 (standardized mortality ratio=0.25, 95% confidence interval, 0.03-0.89). This deficit may have been due to chance, but another possible explanation is the presence of an unidentified confounder. If confounding explains this large deficit of CLL-NHL deaths among workers unexposed to styrene, it is not known if using internal comparison procedures (as done for analyses reported in table 3-3 of the Background document) removed such confounding. The Styrene Expert Panel’s Subgroup Report for Section 3 (page 3, second paragraph) suggests that the fact that “...workers who survive to go from low to high categories will contribute many person-years to low dose groups as they accumulate dose...could account for the very low SMRs seen in the “0” and lowest dose categories.” ***The rationale for this explanation is not clear, and the suggested explanation is implausible.***

The Panel’s justification document emphasizes the inevitable misclassification of exposure to styrene in the epidemiologic studies, repeatedly stating that such misclassification “...would be expected to be nondifferential and to bias any measures of association towards no effect” (page 161). This statement is not necessarily true in analyses of exposure-response in which RRs are calculated for each exposure category, compared to a nonexposed or lowest exposure referent category. More importantly, a presumably underestimated exposure-response association is not tantamount to evidence supporting a true causal relationship. At most, it may be reasonable to argue that lack of an association in a study with an unusual amount of misclassification does not constitute strong evidence against the existence of a true causal relationship, when there are other studies that support such a relationship. In the case of styrene, the other studies do not support such a relationship.

C. Panel’s Lack of Definition on Appropriate Exposure Measure

The Expert Panel asserted that the RPC studies should be discounted because there were a large number of short-term workers. Yet, in the Kogevinas and Wong cohorts combined there were ~6,700 persons exposed to styrene for greater than 10 years, while in the Delzell SBR cohort – which the Panel did cite – the median duration of exposure was 11 years (~8960 persons exposed for greater than 11 years). ***The Panel did not offer any explanation as to how they determined the SBR cohort was adequate but the RPC cohort was not,*** despite the fact there does not seem to be an appreciable difference between the two cohorts in the number of workers exposed for more than 10 years.

If styrene is acting as a non-threshold (genotoxic) carcinogen, as the Subgroup suggests, then risk should be equally increased in short and long-term workers as a function of their cumulative exposure. The theory behind a genotoxic mode of action (MOA) is that the carcinogen causes DNA damage and the risk of developing cancer is proportional to the number of “hits” of the carcinogen on the DNA. This MOA clearly indicates that cumulative exposure is more important than duration of

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exposure as a risk factor for genotoxic carcinogens. The median cumulative exposure in the Delzell SBR cohort was 13 ppm-years (~8962 persons exposed to greater than 13 ppm-years). In the Kogevinas RPC cohort ~30,516 persons were exposed to greater than 75 ppm-years styrene. In the Wong RPC study, ~11870 were exposed to greater than 10 ppm-years and ~7910 of those were exposed to greater than 30 ppm-years. Clearly in both the Wong and Kogevinas RPC studies separately there were more workers exposed to equal or greater cumulative styrene than in the Delzell SBR cohort, yet these studies did not show the effects purported to be shown in the SBR cohort.

It is not the duration of exposure to the carcinogenic agent that matters most, as is suggested by the Subgroup, but rather that there be an adequate follow-up time so that any induced cancer incidence or mortality will be observable. Follow-up time in the RPC workers is clearly comparable to that of SBR workers. In addition, RPC workers had much higher exposure levels than SBR workers, so, if styrene is a human carcinogen, risks should be higher in this industry.

Based on duration of exposure, the RPC studies provide numbers of persons exposed for long periods of time (greater than 10 years) that were comparable to the number in the SBR study. ***The results of the RPC studies cannot be dismissed on the basis that too few persons were exposed long-term.*** Based on cumulative exposure, the RPC studies provide more persons with greater exposure than the SBR cohort. Yet cancer risks are not higher in the RPC industry, and these RPC studies are not supportive of a causal association.

Thus, based on either duration of exposure, as suggested to be most important by the Expert Panel, or cumulative exposure, as suggested to be most important by Drs. Delzell, Teta, Goodman, and Rhomberg in comments submitted to the NTP, the RPC studies provide an equivalent number of workers exposed for more than 10 years and provide more than 30,000 workers with greater cumulative exposures than the SBR studies, and should provide the best assessment of styrene's cancer risk in humans. The RPC studies do not support a causal association.

Summary of Comments on Human Data Conclusions:

- ***As stated by Dr. Delzell, results for styrene and NHL from both studies (Delzell and Kogevinas) are unconvincing. The data do not support a conclusion of "limited evidence of cancer in humans."***
- According to Dr. Delzell, there was no exposure-response relationship for CLL and styrene, but there was for butadiene and CLL; therefore, an analysis of NHL and CLL combined, as done by the Expert Panel, is not appropriate.
- ***In the UAB studies (in the Health Effects Institute report or publications), there were no analyses for exposure-response trends for NHL and styrene or for NHL-CLL combined and styrene; therefore, either the conclusions of the Expert Panel were based on new analyses by the Expert Panel or they have no statistical basis for their conclusion.***

- The Expert Panel wrongfully dismissed a statistically significant deficit in NHL when the unexposed workers were compared in an external comparison (2 observed vs. 8.1 expected; SMR 0.25, 0.03-0.89). While there were higher, but not significant, incidences of NHL among exposed workers than among the unexposed workers, the incidence among the exposed workers was not increased relative to the general population, and according to Dr. Delzell likely represents either chance or an unknown confounding factor.
- The data from the Kogevinas RPC study do not support an increase in NHL. There was no increase in NHL based on average exposure, time since first exposure, cumulative exposure, or duration of exposure.
- As noted in the comments on the draft background document by Dr. Rhomberg, there is no consistent pattern of increases in cancer among styrene workers in different studies. Very few of the differences are statistically significant from unexposed or referent groups. Most of those that are significant are significantly lower than expected, not increased.
- The data from Wong et al. (1994) and Ruder et al. (2004) contribute an additional 20,000+ workers and do not indicate increased cancer risks among RPC workers.
- Overall, there is no evidence of increased cancer among more than 60,000 RPC workers. Evidence for increased NHL among SBR workers is unconvincing and is not supported by higher exposures in RPC workers.

Comments on Expert Panel Conclusions on Studies on Experimental Animals

From the Expert Panel Report:

“Evidence on the carcinogenicity of styrene comes from a number of studies in rats and mice. There are robust studies in male and female mice and rats by oral gavage (NCI 1979a) and inhalation (Cruzan *et al.* 2001, Cruzan *et al.* 1998) routes, along with several other studies more limited in their ability to detect carcinogenic effects because of study design (low doses, short treatment, short study duration, small group size), high early mortality, or limited reporting (tumor diagnosis) (Beliles *et al.* 1985, Brunnemann *et al.* 1992, Conti *et al.* 1988, Jersey *et al.* 1978, Ponomarev and Tomatis 1978).

Sufficient evidence of carcinogenic activity comes from multiple studies in mice exposed to styrene by multiple routes. Styrene induced benign and malignant lung tumors in male and female mice by inhalation (Cruzan *et al.* 2001) and in male mice by oral intubation (NCI 1979a). This is supported by findings of lung tumors in both sexes of mice in studies of more limited design (Ponomarev and Tomatis 1978). There is also the finding in rats of malignant mammary tumors by inhalation (Conti *et al.* 1988) and a small increase in mammary fibroadenoma in a relatively low dose drinking water study (Beliles *et al.* 1985), but mammary tumors were not increased in an adequate inhalation study in the same rat strain exposed for two years (Cruzan *et al.* 1998), limiting the weight given to the mammary tumor findings. In earlier reviews (IARC 1994b, NTP

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2002), sufficient evidence in animals was found for the carcinogenicity of styrene-7,8-oxide."

Comments:

The animal data provide limited evidence of carcinogenicity based on no evidence in rats and limited evidence in mice.

Mice:

The NTP Expert Panel judged that the mouse studies provided sufficient evidence of carcinogenicity based on an inhalation study (Cruzan et al., 2001) and a gavage study (NCI, 1979).

The inhalation study did find increased lung tumors in male and female mice that would be labeled as "clear evidence" if it were an NTP study. In contrast, the NCI study (1979a) found increased lung tumors only in males. The high dose was significantly increased compared to controls and there was a significant trend; the incidences were 0, 12 and 18% at 0, 150 and 300 mg/kg/day, respectively. However, the NTP pointed out they did not have a sufficient number of vehicle treated controls for a historical control comparison (only 1 other study with 20 vehicle-treated males), but relied on historical data from untreated mice at that laboratory. Untreated male mice averaged 12% (32/271), with incidences up to 20% in 2 different studies. The NCI report concluded the increased lung tumors in male mice constituted "suggestive evidence of carcinogenicity." ***The Expert Panel has not provided justification for changing the conclusion of the NCI report from "suggestive" to "clear" evidence.***

The NTP Guidelines say the data are sufficient in animals if there are two or more studies with increased tumors. For styrene, there is only one study in mice that has clear evidence of cancer in mice, and two of 4 others that provide suggestive evidence. ***Overall, the mouse data on styrene should be judged as providing "limited evidence," not "sufficient evidence" as proposed by the Expert Panel.***

Rats:

The NTP Expert Panel noted increased mammary tumors in the Conti et al., 1988 study and increasing trend in the Beliles et al., 1985 drinking water study, but noted that lack of increased mammary tumors in the Cruzan et al., 1998 inhalation study limited the weight given to the mammary tumors. First, the report does not draw a distinction between fibroadenomas, which are the most common in rats, do not progress to malignancy, and do not have a human counterpart, and adenomas/adenocarcinomas which do show progression and are similar to human mammary cancers. In the table below, each dose level (by cumulative lifetime dose) of the 8 chronic studies of styrene in rats is shown with the results of the mammary adenocarcinomas. There are four gavage studies of styrene in F344, Sprague-Dawley and BD-IV rats; none of these increased mammary tumors.

The Expert Panel reported that there was a small increase in fibroadenomas in the Beliles et al., 1985 drinking water study. Note that, due to the limited solubility of

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styrene in water, the daily and cumulative doses were low. The Beliles et al., 1985 drinking water study may suggest increased fibroadenomas in female rats exposed to styrene, but fibroadenomas are not relevant to human risk assessment. Furthermore, the doses were very low compared to other oral and inhalation studies; increased mammary fibroadenomas were not reported in other studies at higher doses. Beliles et al., 1985 found no increase in mammary adenomas or adenocarcinomas (9.4%, 16.7% and 13.3% combined for 0, 125, and 250 ppm, respectively).

Table 1. Malignant mammary tumor results in rats exposed to styrene

Strain	Route of Exposure	Administered Daily Dose	Lifetime dose (g/kg)	Reported Response	Reference
SD	Inhalation	25 ppm	1.9	=	Conti et al., 1988
SD	Inhalation	50 ppm	3.9	=	Conti et al., 1988
SD	Inhalation	100 ppm	7.7	Inc.	Conti et al., 1988
SD	Water	125 ppm	9.9	=	Beliles et al., 1985
SD	Inhalation	50 ppm	11.6	=	Cruzan et al., 1998
SD	Gavage	50 mg/kg/day	13.2	=	Conti et al., 1988
SD	Water	250 ppm	14.9	=	Beliles et al., 1985
SD	Inhalation	200 ppm	15.3	Inc.	Conti et al., 1988
SD	Inhalation	300 ppm	23	Inc	Conti et al., 1988
F344	Gavage (m)	175 mg/kg/3x	42	=	NCI, 1979b
SD	Inhalation	200 ppm	45	=	Cruzan et al., 1998
BDIV	Gavage	500 mg/kg/wk	53	=	Ponomarev, 1978
SD	Gavage	250 mg/kg/day	66	=	Conti et al., 1988
F344	Gavage	350 mg/kg/3x	84	=	NCI, 1978
SD	Inhalation	500 ppm	115	Dec.	Cruzan et al., 1998
SD	Inhalation	600 ppm	115	?	Jersey et al., 1978
SD	Inhalation	1000 ppm	192	=	Jersey et al., 1978
SD	Inhalation	1000 ppm	230	Dec.	Cruzan et al., 1998
F344	Gavage	500 mg/kg/day	264	=	NCI, 1979a
F344	Gavage	1000 mg/kg/day	396	=	NCI, 1979a
F344	Gavage	2000 mg/kg/day	792	=	NCI, 1979a

Conti studies dosed for 12 months; Gavage (m) was 30% b-nitrostyrene; 70% styrene – dose is styrene only; dosed 3 x/week.

The Expert Panel cited increased malignant mammary tumors in female rats in the Conti et al., 1988 study conducted at the Ramazzini foundation facility in Italy. (1.) The Ramazzini Foundation pathologists usually do not subject their pathology slides and diagnoses to outside peer review; however, for the aspartame study, they submitted 75 slides with a variety of diagnoses to an NTP Panel. Three of those slides had tumors designated as mammary adenocarcinomas. In all three cases, the NTP panel voted that they were “fibroadenomas,” not “adenocarcinomas.” Thus the diagnosis of malignant mammary tumors in the styrene study is questionable. (2.) Increased mammary adenocarcinomas were not found in other studies at similar and higher doses. (3.) The incidences of malignant mammary tumors in rats exposed to styrene were within the historical control range for Sprague-Dawley rats (Charles River Database). (4.) At the

highest doses tested by inhalation (Cruzan et al.) there was a significant decrease in mammary tumors.

From the summary of the Background document “An oral gavage study in F344 rats (NCI 1979a) and an inhalation study in Sprague-Dawley rats (Cruzan *et al.* 1998) were the most robust and most completely reported carcinogenicity studies. Neither study showed an increase in tumor incidences in styrene-exposed rats, although Sprague-Dawley rats exhibited a negative trend in pituitary and mammary gland tumors and a positive trend for testicular interstitial-cell tumors.” These two studies cover a very large dose range and two routes of exposure (oral and inhalation). Given the principles of dose-response, questionable increases in mammary tumors at relatively low doses should be compared to the two quality studies. ***The conclusion (as reached by IARC, 2002; Harvard, 2002; EU, 2007) should be that there are no increased tumors in rats from exposure to styrene.***

Overall Conclusion from Animal Studies

Based on 8 chronic studies, styrene does not cause increased tumors in rats. Lung tumors were increased in one mouse study by inhalation; 2 of 4 gavage studies of styrene in mice provided suggestive evidence of increased lung tumors. Gavage administration of styrene-7,8-oxide did not produce increased lung tumors in mice. ***Overall, the animal data on styrene provide limited evidence of carcinogenicity.***

Mechanistic Concerns

Metabolic conversion to styrene-7,8-oxide occurs to a greater extent in rats at non-tumorigenic exposures and is unrelated to tumor formation. Tumors occur at sites high in cyp2f2 and occur following extensive cytotoxicity and regenerative hyperplasia. A thorough discussion of the cyp2f2-mediated mouse lung tumor is attached. Rats do not have sufficient levels of cyp2f4 to cause cytotoxicity in lung and do not form lung tumors. Humans have very low levels of CYP2F1 and very little ability to metabolize styrene in lungs. Therefore, the mode of action for mouse lung tumors from styrene exposure indicates these are not relevant for human risk.

The animal studies of styrene, when taken as a whole, do not provide evidence of increased mammary tumors, nor increased prolactin in rats. Thus a very slight elevation of prolactin within the normal range does not provide “Support for a mechanism of mammary tissue as a site...”.

From the Expert Panel Report:

“There are at least two reasonable, literature-supported, and scientifically valid mechanisms for styrene-induced cancer. These mechanisms are not mutually exclusive. These mechanisms supported the panel’s decision to recommend listing styrene in the Report on Carcinogens.

- Styrene is genotoxic to human lymphocytes through sister chromatid exchange processes and chromosomal aberrations (e.g., micronuclei and aneuploidy). Styrene is mutagenic through formation of styrene-7,8-oxide. Cyp2f2 in mouse

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lung cells efficiently oxidizes styrene to styrene-7,8-oxide. In addition, CYP2F1, CYP2A13, and CYP2S1 are expressed in many extrahepatic organs, and their known catalytic activity with styrene supports a plausible mechanism by which styrene is bioactivated to styrene oxide in many human tissues. Styrene-7,8-oxide forms multiple DNA adducts, primarily O⁶ and N7 of guanine, in cultured human lymphocytes and embryonal lung fibroblasts. In addition, lymphocytes from styrene-exposed workers possess the same DNA adducts.”

Comment:

The statement that the two possible modes of action (genotoxicity through styrene-7,8-oxide, and cyp2f2-mediated cytotoxicity) are not mutually exclusive is unsupported opinion. No facts were provided to support that conclusion. We have provided comments on the role of each of these proposed MOAs for styrene-induced cancer.

Genotoxicity: The evidence of genotoxicity is much less straightforward than proposed in the evaluation. Low levels of protein and DNA adducts have been reported in rats, mice and humans exposed to styrene. Styrene in general was not mutagenic in 14 standard Ames tests. Styrene causes increased chromosomal aberrations (CAs), micronuclei (MN) and sister chromatid exchange (SCE) *in vitro*. However, styrene did not cause increased MN in 5 of 6 studies or 11 of 12 CA assays in experimental animals. Mixed results for CA and MN (about half positive) have been reported in workers in industries where there is exposure to styrene. Interestingly, more of the “positive” studies have been reported more recently, when workplace exposures have been reduced significantly. This is an apparent antidose-response, or some factor(s) other than styrene is responsible for the recent positive results.

Furthermore, there are several compounds that also cause mouse lung tumors, and not rat lung tumors, that do not form an epoxide side-chain and have a negative genotoxicity profile. The fact that styrene may be positive in some genotoxicity tests does not automatically mean it has a genotoxic mode of action. In the attached description of the proposed MOA is an assessment of genotoxicity of these compounds.

From the Expert Panel Report:

- “Styrene is potentially carcinogenic to lung tissues because sufficient evidence exists in animals for bioactivation to the mutagenic epoxide, and tumors are produced within the same sites where P450s are expressed, and DNA adducts are also identified.”
- “Styrene bioactivation to the 7,8-epoxide or 3,4-epoxide is cytotoxic to mouse lung cells. Specifically, the 3,4-epoxide could form 4-vinylphenol which could be oxidized to dihydrodiols and quinones by P450 enzymes and produce cytotoxicity through reactive oxygen mechanisms. Subsequent cellular proliferation and clonal expansion would then constitute an epigenetic mechanism. Recent evidence suggests that mice may be especially sensitive to lung carcinogenesis through this mechanism, and it may be operative in humans as well.”

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Comment:

There is no evidence that styrene metabolism to styrene-7,8-oxide is related to the organ selectivity of styrene toxicity and tumors. Styrene cytotoxicity in rats and mice occurs in tissues that are high in cyp2f enzymes; these are nasal olfactory epithelium in rats, and lung Clara cells and nasal olfactory epithelium in mice. Experiments in mice have shown that the cytotoxicity in both Clara cells and nasal olfactory epithelium is inhibited by inhibition of cyp2f2 by 5-phenyl-1-pentyne, and is not inhibited by inhibition of cyp2e1 or in cyp2e1-knockout mice exposed to styrene. Tissues high in cyp2f produced primarily ring-oxidized metabolites and R-styrene oxide (whereas tissues high in cyp2e1 produce mostly S-styrene oxide). The ring-oxidized metabolites are toxic at much lower levels than styrene or styrene oxide and their toxicity is also inhibited by inhibiting cyp2f2 metabolism.

Although styrene-7,8-oxide is produced extensively in mouse lung at 40 ppm (tumorigenic), greater amounts occur in rat lungs at non-tumorigenic doses up to 1000 ppm. There is no organ selectivity for DNA adducts; DNA adducts are no greater in mouse lung than in rat lung, nor in mouse lung than in mouse liver. Gavage administration of styrene-7,8-oxide to mice resulted in as much styrene oxide being present in the terminal bronchioles as inhalation of 40 ppm styrene, but did not result in cytotoxicity or increased lung tumors. These observations, detailed in comments on the draft Styrene Background Documentation previously submitted, provide adequate evidence that increased lung tumors in mice exposed to styrene by inhalation are NOT related to the production of styrene-7,8-oxide; therefore, neither the tumorigenic classification of styrene oxide nor its genotoxicity profile are relevant for classifying the carcinogenic potential of styrene.

Two PBPK models which include lung metabolism have evaluated the levels of styrene metabolites in the lungs of mice, rats and humans. Both indicate that humans have a much lower level of styrene metabolites in lung cells than do mice (less than 1%). The Sarangapani et al. (2002) model estimates that the maximal level of styrene oxide in human lung at saturation (200 ppm exposure; 10 times the ACGIH exposure limit) is less than 3% of the level present at the EDL₁₀ for mouse lung tumors.

Summary:

Metabolic conversion to styrene-7,8-oxide occurs to a greater extent in rats at non-tumorigenic exposures and is unrelated to tumor formation. Tumors occur at sites high in cyp2f2 and occur following extensive cytotoxicity and regenerative hyperplasia. Rats do not have sufficient levels of cyp2f4 to cause cytotoxicity in lung and do not form lung tumors. Humans have very low levels of CYP2F1 and very little ability to metabolize styrene in lungs; levels of styrene metabolites are only a small fraction of the tumorigenic level in mouse lung. Therefore, ***the mode of action for mouse lung tumors from styrene exposure indicates these are not relevant for human risk.***

From the Expert Recommendation:

- "Support for a mechanism of mammary tissue as a site includes limited human evidence for elevated prolactin levels from styrene-exposed workers."

Comment:

There is no evidence of clinically elevated prolactin in styrene-exposed workers. The Styrene Background Document that served as the basis for this conclusion on partially quoted from the NTP CERHR evaluation of styrene. The full quotation is "Evidence from studies conducted in occupational settings suggest that exposure of women to styrene is associated with slightly increased levels of prolactin and possible depletion of peripheral blood dopamine metabolizing enzyme activities when compared to levels in women not occupationally exposed to styrene. However, the clinical relevance of these effects is uncertain because: (1) the average elevation in prolactin concentrations in blood serum was small and within the normal range of blood serum values and (2) menstrual function and other reproductive endpoints were not evaluated in these studies. SIRC submitted written comments on the NTP CERHR Report and proposed that these findings are of no relevance to human health (see Public Comments in Appendix III). Two studies in rats failed to find any effect of exposure to styrene on prolactin concentrations in serum. Maximum exposure levels of styrene used in the rat studies were 1500 ppm by inhalation for 5 days and 200 mg/k body weight/day injected subcutaneously for 7 days."

The RoC Background Document further cites a paper by Harvey that proposes that elevated prolactin is a risk factor in human breast cancer. In subsequent papers (Harvey et al., 2008; Tworoger et al., 2006) found an increased risk of breast cancer among women with prolactin above 21 ng/mL compared with those below 11 ng/mL. In the Luderer study of styrene-exposed workers, very few had prolactin levels above 21 ng/mL estimated on the mean and standard deviation provided by Luderer et al.

The animal studies of styrene, when taken as a whole, do not provide evidence of increased mammary tumors, nor increased prolactin in rats. Thus a very slight elevation of prolactin within the normal range does not provide "Support for a mechanism of mammary tissue as a site..."

The details of these concerns follow.

2 - Significant Scientific Procedural Flaws on the Part of the Expert Panel

During the course of the July 21-22 Expert Panel meeting, the Epidemiology Subgroup reviewed data in a study by Delzell et al (2006) in styrene-butadiene rubber workers, which reported that the data were inconclusive regarding the possibility of a link between styrene exposure and cancer. However, the Epidemiology Subgroup reanalyzed these data and concluded that they supported a finding of increased incidence of cancer. SIRC observers at the Expert Panel meeting recall that the Epidemiology Subgroup itself characterized this as a "reanalysis" of the Delzell data.

In the Epidemiology Panel's reanalysis, increased NHL and NHL-CLL combined, based on the data found in Delzell's paper, was cited as the Expert Panel's key finding leading to its recommendation for classifying the human data as "limited evidence;" increased total lymphomas (but not NHL) by one measure of exposure in the Kogevinas et al. study was cited as supporting evidence.

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The Epidemiology Panel therefore apparently developed its own new data analysis either prior to, or during the course of, the Panel meeting. SIRC's assessment of the situation has been supported by Dr. Elizabeth Delzell herself, who SIRC asked to review the NTP Background Document and Panel recommendation. Dr. Delzell has confirmed that there were no analyses for exposure-response trends for NHL and styrene or for NHL-CLL combined and styrene in the original study; therefore, either the conclusions of the Expert Panel were based on new analyses by the Expert Panel or there is no statistical basis for the Panel's conclusion.

Shortly after the Expert Panel meeting, SIRC contacted NTP requesting the supporting analyses developed by NTP and/or the Epidemiology Subgroup that would provide the necessary justification for the Panel's conclusion of a cancer finding, as this finding was not included in the paper by Delzell et al. This new analysis, assuming it exists, has not been made available to the public, to date, for comment, has not been externally peer reviewed, and was not cited in the Styrene Background Document.

SIRC has, on several occasions, written to NTP regarding the fact that it has commissioned an independent Blue Ribbon Panel, made up of five internationally respected epidemiologists with impeccable credentials, to review the full body of styrene epidemiology data, including the Expert Panel's reanalysis and conclusions; and that this Panel is expected to provide a report directly to NTP by December 22, 2008. SIRC requested that the October 23, 2008 deadline for comments on the Expert Panel recommendation be extended to December 31, 2008, in order for NTP to receive this report as a public comment and give it thoughtful consideration.

The apparent lack of supporting analysis, as well as the lack of external review of this new analysis of the styrene data, calls into question the validity of the Panel's recommendation, as well as NTP's transparency in the review process – particularly since this study is one of only a few key studies cited in supporting the styrene classification and listing.

Should the Panel's reanalysis of the Delzell data prove to be scientifically unsupportable – or if there was no supporting analysis – this fact alone would invalidate the Panel's entire efforts.

Yet, SIRC believes this procedural error could be easily addressed by extending the comment period and then fully and thoughtfully considering the Blue Ribbon Panel report. This would provide the appropriate external scientific peer review of the Panel's reanalysis of the Delzell data. To date, however, NTP has denied SIRC's request to resolve this procedural error with this timely and simple proposal by simply allowing an extended comment period.

3 - Ongoing Frustrations with Public Participation Process

SIRC has submitted large amounts of significant data and comments to NTP – virtually all of which has gone unaddressed by NTP staff, its contractor, and the Expert Panel. We thus are extremely concerned because the documentation that has been developed on styrene, as well as the report of the Expert Panel, does not accurately reflect the full scientific database on styrene, or a balanced assessment of those data. SIRC further is concerned that the subsequent review of the Expert Panel's styrene listing recommendation by the Interagency Panel and NIEHS's Board of Scientific

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Counselors will not provide a straightforward opportunity to address either the scientific or procedural concerns we have with the NTP *RoC* process for styrene.

In response to NTP *Federal Register* notices, SIRC has submitted substantial amounts of data for NTP's reference, starting in 2004 to inform NTP as it drafted its styrene Background Document, when NTP first announced that styrene was being considered for inclusion in the *RoC*. SIRC subsequently submitted significant comments on the draft Background Document – in particular because we found the document to be extremely unbalanced from a scientific perspective, and lacked a discussion of many of the key studies on styrene. SIRC again was disappointed to see that virtually none of its comments on the Background Document had been addressed in the final version of the Document, which was completed in September 2008.

NTP convened a Styrene Expert Panel Meeting on July 21-22, 2008. This meeting was attended in person by numerous representatives of SIRC, as well as other styrene-using industry associations. Each of these groups also presented oral comments to the Expert Panel, which reflected their written comments expressing concerns with the NTP's draft Styrene Background Document. SIRC and its sister organizations presented substantial scientific data, both written and oral, which we believe – given a thoughtful, balanced and scientifically grounded review – indicate that styrene should not be considered a concern as a human carcinogen. SIRC was therefore surprised and disappointed that these data were not discussed during the course of the Panel meeting, nor was there any evidence that they were considered as part of the Panel's review that ultimately led it to recommend styrene be listed as "reasonably anticipated to be a human carcinogen."

Based on SIRC's experiences with the styrene *RoC* review thus far, it has become clear that the new *RoC* process is not adequate to address substances with databases as complex as styrene's. For example, it seems inappropriate and counter-intuitive for NTP to finalize the Background Document prior to the close of the public comment period regarding the Expert Panel's listing recommendation and scientific justification. The Expert Panel meeting is intended to provide peer review of the Background Document, therefore consideration of the public's comments on the Expert Panel's review seems to be an essential step before finalizing the Background Document. Yet, in the case of styrene, this document was finalized weeks before the deadline for comments on the Panel recommendations.

The Styrene Background Document is a public document that will become a fundamental federal reference document on the carcinogenic potential of styrene, yet it does not begin to reflect the full nature of the database on styrene. It is unrealistic to assume that the public – academic, regulatory, or otherwise – will look at SIRC's comments in future, once the NTP styrene process has been finalized. Consequently, the Background Document will remain the "final word" in the public's mind. And in the context of the subsequent steps of the review of the Expert Panel's recommendations regarding styrene, SIRC has received little assurance that our extensive comments and communications regarding NTP's procedural errors (i.e. the introduction of new data and analysis during the Expert Panel meeting, and completion of the Background Document before the close of the comment period) will be relayed to, discussed by, or considered during the subsequent steps in the review of styrene's proposed listing, during internal NIEHS review, by the Interagency Panel, or by the Board of Scientific Counselors.

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NTP's next step in the review process for the RoC calls for the preparation of a Substance Profile that will "summarize the scientific information that supports the recommendation..." The Substance Profile will be based on the Background Document, and errors in the Background Document should be corrected, if even for the sake of conformity with the Substance Profile.

As we noted in our letter of October 21 to Dr. Samuel Wilson, it appears that NTP's approach to public input has been "Receive, Post, but Do Not Consider." SIRC does not believe that simple posting of our comments in the public docket constitutes a scientifically appropriate approach as a component of the RoC process. Yet, this appears to be all that the NTP process and expedited schedule provide.

4 - Lack of Scientific Balance in the RoC Process

Rather than working to ensure that all the available data had been given a thoughtful, scientifically balanced assessment, it appeared to SIRC observers at the July 21-22 Expert Panel meeting that the Panel came to the meeting with a predisposition that styrene would be classified and listed. This view was reinforced by the fact that the Panel's chair on at least two occasions needed to be reminded that the Panel did, in fact, have the option to recommend that styrene not be classified.

NTP's disregard of SIRC's and other industry comments – which laid out valid, scientifically supportable arguments for why styrene should not be classified as a carcinogen – were not reflected in NTP's Background Document (apart from study citations in the reference list), and were not publically discussed by the Expert Panel, despite the Panel having been presented this information both orally and in writing.

Essentially, from the observations of SIRC attendees at the Panel meeting, the Panel worked to identify positive data in an effort to support a classification proposal, and ignored or dismissed the wealth of negative data. Based on the way in which the Expert Panel apparently ignored an entire body of negative evidence, the NTP review process does not appear to follow key fundamental "weight of the evidence" principles which are commonly used in developing hazard assessments.

SIRC certainly acknowledges that the Panel has the right to reach its own conclusions regarding the data, but it would appear that the Styrene Expert Panel either was not provided with, or chose to ignore, large amounts of scientific data which should have been considered in order for the Panel to truly have provided a weight of the evidence assessment. Surely it was Congress' intention in mandating the *Report on Carcinogens* that substances be given a balanced review, and that NTP should make every effort to lay out the complete database on a substance when considering something as fundamental as a substance's ability to cause cancer.

5 - Conclusion

SIRC asks that NTP give careful consideration to its comments from a perspective of balanced, inclusive science. In particular, the agency should validate its position relative to the reanalysis of the Delzell data, to ensure that it is scientifically supportable. SIRC once again points out the imperative need for NTP to obtain valid external peer review of the Expert Panel's new analysis prior to proceeding with the

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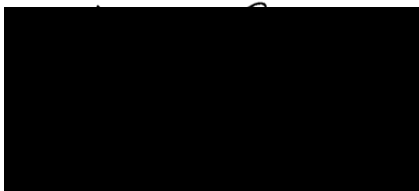
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styrene RoC review process, and again urges NTP to extend the comment period to December 31, 2008, in order to have the benefit of the report of the independent styrene Blue Ribbon Epidemiology Panel which SIRC has commissioned.

The listing of styrene in the RoC could have a profoundly negative impact for the styrene industry, as well as consumers of thousands of products currently made using styrene. SIRC continues to believe that NTP's currently proposed listing of styrene is not grounded in a thorough and balanced review of the available scientific data on styrene. This absence of balance in the NTP process does a disservice to the American public which the RoC is intended to enlighten.

We ask again that NTP confirm the need for external peer review of the reanalysis of the Delzell study, extend the comment period to allow the Blue Ribbon Panel report to be submitted and considered as a public comment, and that NTP correct the failures of its contractor and the Expert Panel by committing to consider all of the relevant styrene research in its further deliberations.

Very truly yours,



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cc: Dr. Samuel Wilson, NIEHS/NTP

Attachments:

These comments were submitted with the following two document attachments, in the form of additional electronic files, which are intended to be included in the public docket with this comment letter, and which are referenced herein:

- *Comments on the RoC Background Document for Styrene and Related Documents*, by E. Delzell
[Electronic File Name: SIRC Comments Attachment A 10-23-08]
- *Mouse Specific Lung Tumors from cyp2f2-Mediated Cytotoxic Metabolism: An Endpoint/Toxic Response Where Data from Multiple Chemicals Converge to Support a Mode of Action*, by Cruzan et al.
[Electronic File Name: SIRC Comments Attachment B 10-23-08]